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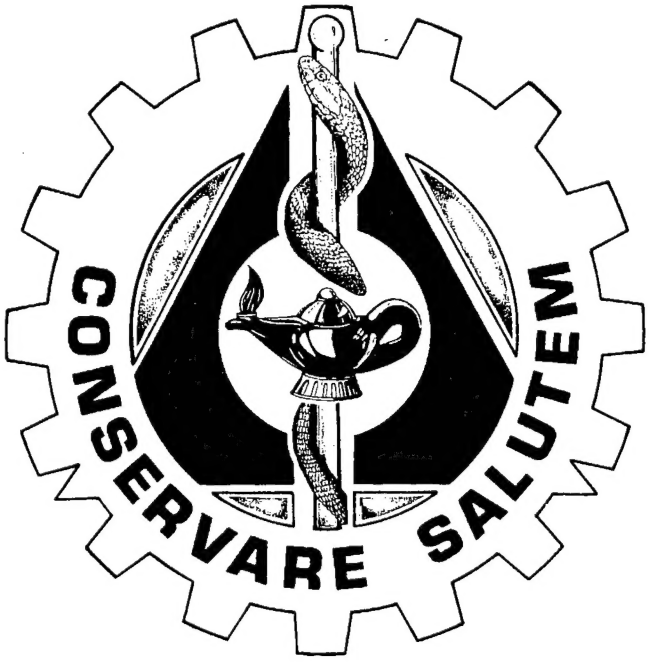

TOXICOLOGICAL STUDY NO. 75-51-0805-91
DERMAL PENETRATION OF THE CANDIDATE INSECT REPELLENT
AI3-37220 IN SWINE AND RABBITS
OCTOBER 1996

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Readiness Thru Health

U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) lineage can be traced back over a half century to the Army Industrial Hygiene Laboratory which was established at the beginning of World War II under the direct jurisdiction of The Army Surgeon General. It was originally located at the Johns Hopkins School of Hygiene and Public Health with a staff of three and an annual budget not to exceed three thousand dollars. Its mission was to conduct occupational health surveys of Army-operated industrial plants, arsenals, and depots. These surveys were aimed at identifying and eliminating occupational health hazards within the Department of Defense's (DOD) industrial production base and proved to be extremely beneficial to the Nation's war effort.

Most recently, the organization has been nationally and internationally known as the U.S. Army Environmental Hygiene Agency (AEHA) and is located on the Edgewood area of Aberdeen Proving Ground, Maryland. Its mission had been expanded to support the worldwide preventive medicine programs of the Army, DOD and other Federal agencies through consultations, supportive services, investigations and training.

On 1 August 1994, the organization was officially redesignated the U.S. Army Center for Health Promotion and Preventive Medicine and is affectionately referred to as the CHPPM. As always, our mission focus is centered upon the Army Imperatives to that we are optimizing soldier effectiveness by minimizing health risk. The CHPPM's mission is to provide worldwide scientific expertise and services in the areas of:

- Clinical and field preventive medicine
- Environmental and occupational health
- Health promotion and wellness
- Epidemiology and disease surveillance
- Related laboratory services

The Center's quest has always been one of customer satisfaction, technical excellence and continuous quality improvement. Our vision is to be a world-class center of excellence for enhancing military readiness by integrating health promotion and preventive medicine into America's Army. To achieve that end, CHPPM holds everfast to its core values which are steeped in our rich heritage:

- Integrity is our foundation
- Excellence is our standard
- Customer satisfaction is our focus
- Our people are our most valuable resource
- Continuous quality improvement is our pathway

Once again, the organization stands on the threshold of even greater challenges and responsibilities. The CHPPM structure has been reengineered to include General Officer leadership in order to support the Army of the future. The professional disciplines represented at the Center have been expanded to include a wide array of medical, scientific, engineering, and administrative support personnel.

As the CHPPM moves into the next century, we are an organization fiercely proud of our history, yet equally excited about the future. The Center is destined to continue its development as a world-class organization with expanded preventive health care services provided to the Army, DOD, other Federal agencies, the Nation, and the world community.

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Hubert L. Snodgrass, John T. Houpt, Patricia A. Beall

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The study was performed to measure the dermal absorption of the candidate insect repellent AI3-37220 in swine and rabbits. Percutaneous absorption of ^{14}C-labeled AI3-37220 in swine measured 8 percent of the applied dose through 7 days. Urinary excretion was the major elimination pathway for absorbed chemical. No significant tissue deposition was observed. In rabbits, dermal absorption measured 70 percent in animals where the substance was washed off after 24 hours. When left on the rabbit skin for 7 days, absorption totaled 76 percent. No bioaccumulation was observed in rabbit tissues. Within the intended use of AI3-37220 as a topical insect repellent, skin absorption in humans would be expected to be less than 8 percent of an applied dose. Evaporation of the material is likely to exceed 20 percent of the applied dose within the first 24 hours.

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Dermal Penetration of the Candidate Insect Repellent
AI3-37220 in Swine and Rabbits
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Author

Hubert L. Snodgrass

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d) (1) (A), (B) or (C).

Organization : Department of Defense , Armed Forces Pest Management Board (AFPMB)

Organization's Agent: Donald P. Driggers, COL, MSC, USA Date: November 7, 1996

Executive Director, AFPMB, Study Sponsor
Title

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Submitted By: U.S. Army Center for Health Promotion
and Preventive Medicine
Toxicology Directorate
ATTN: MCHB-DC-TTE
Aberdeen Proving Ground, Maryland 21010-5422

Prepared By:

Hubert L. Snodgrass — 14 December 1996
Biologist — Date

Approved By:

LeRoy W. Metker — 14 Nov 1996
Program Manager — Date
Toxicity Evaluation

DEPARTMENT OF THE ARMY
U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO
ATTENTION OF

EXECUTIVE SUMMARY
TOXICOLOGICAL STUDY NO. 75-51-0805-91
DERMAL PENETRATION OF THE CANDIDATE INSECT REPELLENT AI3-37220 IN SWINE AND RABBITS
OCTOBER 1996

1. PUPOSE. The study was conducted to determine the absorption of AI3-37220, 1-(3-cyclohexene-1-ylcarbonyl)-2-methylpiperidine, when applied to the skin of swine and rabbits so that potential hazards to man could be predicted.

2. FINDINGS. Percutaneous absorption of radiolabeled (^{14}C) AI3-37220 in swine measured 8 percent of the applied dose through 7 days. Nearly all of the absorption occured within the first 48 hours. Urinary excretion was the major elimination pathway for absorbed AI3-37220. No significant tissue deposition of radiocarbon was observed. In rabbits, dermal absorption measured 70 percent in animals where the substance was washed-off after 24 hours. When left on the rabbit's skin for 7 days, absorption totaled 76 percent. It was greatest during the first 24 hours as measured by urinary excretion of radiocarbon. No bioaccumulation of AI3-37220 was recorded for any tissue system monitored in rabbits.

3. CONCLUSIONS. The candidate insect repellent AI3-37220 is minimally absorbed in swine following topical application at a rate of 0.5 mg/cm^2. In rabbits, marked absorption occurs through 1 week. Urinary excretion is the primary elimination pathway of absorbed chemical. Metabolic elimination of AI3-37220 (or its metabolites) is rapid, occurring within 24 hours of absorption. No potential for bioaccumulation has been demonstrated in animals following exposure at 0.5 mg/cm^2. Within the intended use of AI3-37220 as a topical insect repellent, skin absorption in humans would be expected to be less than 8 percent of the applied dose. Evaporation of AI3-37220 from the skin surface is likely to exceed 20 percent of the applied dose within the first day of exposure.

TABLE OF CONTENTS

DEPARTMENT OF THE ARMY
U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO
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TOXICOLOGICAL STUDY NO. 75-51-0805-91
DERMAL PENETRATION OF THE CANDIDATE INSECT REPELLENT AI3-37220 IN SWINE AND RABBITS
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1. REFERENCES. See Appendix A for a listing of references.

2. AUTHORITY.

a. Letter, Department of Defense, Armed Forces Pest Management Board, 18 April 1988, subject: Arthropod Repellent Program, Part VI, Decision.

b. Memorandum of Understanding between the U.S. Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Management Board; and the U.S. Department of Agriculture, Agricultural Research Service, subject: Biological and Toxicological Testing of Pesticides, effective 7 October 1987.

3. PURPOSE. The study was conducted to determine the absorption of AI3-37220, 1-(3-cyclohexene-1-ylcarbonyl)-2-methylpiperidine, when applied to the skin of swine and rabbits so that potential hazards to man could be predicted.

4. GENERAL.

a. AI3-37220 is a candidate insect repellent, first synthesized by the U.S. Department of Agriculture (USDA). In efficacy tests by both the U.S. Army and the USDA, it equals or exceeds the repellency of DEET, the current issue repellent provided to the military (reference 1).

b. The acute toxicity of AI3-37220 has been earlier reported (reference 1). The substance is moderately toxic by the oral route having an approximate lethal dose in rats of 1270 mg/kg. The technical material produces mild irritation to the eyes but only slight irritation to the skin.

Use of company names does not imply endorsement by the U.S. Army, but is intended only to assist in identification of a specific product.

AI3-37220 is not a skin sensitizer in animals nor is it photoactive. Essentially all *in vitro* and *in vivo* mutagenicity assays performed with the repellent were negative. Inhalation of the saturated vapor for 8 hours had no detrimental effects in rats. AI3-37220 did not produce skin sensitization in human subjects during a prophetic patch test. Human participants in laboratory and field trials reported no adverse effects following application of the repellent, except for an occasional warming sensation on the skin.

c. The present study was designed to quantitate, where possible, that portion of topically applied AI3-37220 penetrating the skin and its fate within the body once absorbed. For tracking purposes the material was radiolabeled using carbon-14 (^{14}C). The appearance of ^{14}C in urine and/or in tissues collected at necropsy was used as a measure of percutaneous absorption. Swine were used as a human surrogate because of similarities in absorption kinetics and metabolic treatment of xenobiotics. Rabbits were also used because of their known elevated absorption potential compared to other mammalian species, including man.

5. METHODS.

a. Materials. Nonradioactive (cold) AI3-37220 [1-(3-cyclohexene-1-ylcarbonyl)-2-methylpiperidine] was synthesized and provided by Dr. Terrence McGovern, USDA. It was identified as AI3-37220f, and had a chemical purity of >99 percent as measured by gas chromatography. Radiolabeled AI3-37220 (cyclohexene-1,2,6-^{14}C) was purchased from DuPont, NEN Research Products, Boston, Massachusetts. It had a specific activity of 3.93 mCi/mM and a radiochemical purity of greater than 97.5 percent, as certified by the manufacturer. For treatment in swine, a solution was prepared by combining cold and radioactive AI3-37220 in acetone such that a single dose volume of 1.0 mL contained 50 mg of AI3-37220 and 5 μCi of radioactivity. For rabbits, a similar solution was prepared but the single dose volume was 0.2 mL and contained 10 mg of AI3-37220 and 5 μCi of radioactivity.

b. Animals.*†

(1) Six Yorkshire Cross, SPF, neutered male swine, were obtained from Buckshire Corporation, Perkasie, Pennsylvania. The mean (n=6) body weight at testing was 9.56 kg ($\pm$2.31). Upon receipt, pigs were housed in individual metabolism cages where they resided

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* In conducting the studies described herein, the investigators adhered to the 'guide for the Care and Use of Laboratory Animals," U.S. Department of Health, Education ans Welfare Publication No. (NIH) 85-23, 1985.

† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

throughout the 7-day acclimation and 7-day test periods. A standard environment (72 °F; 40 percent RH) and photoperiod (12:12 hr) were maintained. The diet, Purina Lab Porcine Grower® was limited during the first 72 hours of animal receipt to prevent scouring. Thereafter, a ration of about 2 lb/day was provided. Drinking quality water was available *ad libitum*.

(2) Twelve male New Zealand white rabbits were obtained from Hazelton Research Products, Denver, Pennsylvania. The mean (n=12) body weight at testing was 3.28 kg (±0.34). Animals were housed in individual stainless steel metabolism cages throughout the study. The environment was maintained at 68-70 °F, a relative humidity of 40 percent, and 12:12 hour photoperiod. A laboratory diet of Purina Certified Rabbit Chow 5322® and drinking quality water were available *ad libitum*.

c. Experimental Procedure.

(1) Pigs were weighed and their backs were clipped 48 hours before treatment. Each back was then gently washed with mild soap and rinsed with tap water. For percutaneous (p.c.) treatment, a 100 cm^2 area of the animal's back was demarcated with petrolatum to confine the test solution while the vehicle (acetone) evaporated. Each animal received 1.0 mL of the test substance (50 mg AI3-37220; 5 μCi radioactivity) applied to a 100 cm^2 area of the back. This equalled a dose rate of 0.5 mg/cm^2. Two additional 1 mL doses were dispensed into a volumetric flask using the same syringe. These control specimens were diluted with methanol, refrigerated and later analyzed for ^{14}C-label to confirm the actual dose delivered.

(2) Rabbits were weighed and their backs were clipped 24 hours before AI3-37220 treatment. A 20 cm^2 of the back was demarcated with petrolatum. Each animal received 0.2 mL of the test substance (10 mg AI3-37220; 5 μCi radioactivity) applied to a 20 cm^2 area of the back. The dose rate equalled 0.5 mg/cm^2, the same as that used in swine. Two additional doses were dispensed into a flask containing methanol for counting as a dose check.

(3) Following percutaneous (p.c.) application of the test substance, the treatment area was covered with a nonocclusive patch (reference 2). The patch consisted of a self-adhering foam ring (Reston®) which bordered the site. Following application of the test substance, the

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open center was then covered with a gauze pad sandwiched between two layers of window screen. This resulted in an air space of about 1.5 cm between the skin surface and the nonocclusive covering. The ensemble was held in place by wrapping tape (Elastoplast®) around the edges of the patch and the animal's trunk. Following treatment, each animal was returned to its metabolism cage for the separate collection of excreta.

(4) Twenty-four hours after p.c. application, each protective patch was removed, placed in a plastic bag, and frozen. In all of the pigs and one-half of the rabbits (6) the application site was swabbed with gauze sponges saturated with a mild soap solution. It was then rinsed with tap water, wiped dry and wiped again with gauze moistened with methanol. A fresh protective covering was applied to each animal which remained in place for the remainder of the test. All of the gauze sponges and rinsates were collected in polypropylene bottles and stored frozen for later radiocarbon analysis. In the remaining six rabbits, the test material was not washed off but remained on the animals's back through 7 days.

(5) Urine was collected 24 hours after animal treatment, and daily thereafter through the 7-day study. The volumes were measured and recorded. Immediately upon collection, aliquots of urine, 0.5 mL each, were combined directly with PCS® II scintillation cocktail using an autopipette. Samples were refrigerated until analyzed at the end of the study. Feces were also collected from each animal but due to a freezer malfunction their utility could not be assured.

(6) At the end of the study, pigs were euthanized by intravenous barbiturate overdose. The protective patch and tape bindings were removed and stored as above. The application site was again washed, as described above. The entire skin area which had received the test substance, including about 3 cm adjacent to the test site, was excised and immediately placed in methanol. Major organs including brain, heart, kidneys, liver, lungs, spleen, adrenal glands, thyroid gland and urinary bladder were removed intact and weighed. Samples of each organ (about 0.5 g), in addition to skin, bone, bone marrow, fat, skeletal muscle, and blood were collected and quick-frozen until analyzed for residual ^{14}C.

d. Analytical Methods.

(1) Urine specimens, 0.2 mL in 15 mL of PCS II scintillation cocktail, were analyzed for radiocarbon using a Beckman®, Model 9000, liquid scintillation counter (LSC).

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(2) Tissue samples (0.3-0.6 g) were oxidized to $^{14}CO_2$ using a Packard®, Model 360, biological materials oxidizer. The trapped radiolabel was measured by a LSC. Skin from the application site, the nonocclusive patches and bindings, and gauze sponges used for washing the application site were extracted in methanol. Containers were agitated for 36 to 48 hours using a laboratory platform shaker. For analysis, 0.5 mL aliquots of the methanol extracts were added directly to the scintillation cocktail (PCS II) and counted.

(3) Additional samples, taken at the time of animal treatment as a dose check, were diluted with methanol and added to PCS II for counting. The actual dose received by each animal was later adjusted based on the results of these measurements.

6. RESULTS.

a. Table 1 provides a summary of the daily urinary excretion of ^{14}C following dermal application of ^{14}C-labeled AI3-37220 to swine. About 7 percent of the substance was excreted within the first 48 hours, the remaining 1 percent being recovered through the next 4 days. Dermal absorption, as measured by urinary excretion of the labeled fraction, totaled 8.38 percent of the applied repellent. Data for urinary excretion of AI3-37220 appears in Appendix B.

TABLE 1. MEAN (n=6) DAILY URINARY EXCRETION OF ^{14}C FOLLOWING A SINGLE TOPICAL APPLICATION OF ^{14}C-LABELED AI3-37220 TO SWINE

Exposure Duration	Dose (mg)	Day 1	2	3	4	5	6	7	Total
		Percent of Applied Dose (±SD)							
24 hrs	50	4.20 ±0.87	3.06 ±0.28	0.53 ±0.12	0.19 ±0.10	0.17 ±0.29	0.23 ±0.43	0.00 ±0.00	8.38 ±0.98

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b. Total recovery (mass balance) of ^{14}C from all sources following a single application of labeled AI3-37220 to swine was 98 percent. This is summarized in Table 2. The majority of the material (83 percent) was recovered from the application site 24 hours after application, either from the skin surface or from the protective patch. Data for total recovery of labeled AI3-37220 appears in Appendix C.

TABLE 2. MEAN (n=6) TOTAL RECOVERY OF ^{14}C IN SWINE THROUGH 7 DAYS FOLLOWING A SINGLE TOPICAL APPLICATION OF ^{14}C-LABELED AI3-37220

Exposure Duration	Dose (mg)	Urine	24 hr Wash	24 hr Patch	7 da Patch	7 da Skin Appl Site	Total
		Percent of Applied Dose (±SD)					
24 hrs	50	8.38	63.36	19.57	3.85	2.60	97.76
		±0.98	±9.97	±8.83	±0.93	±0.49	±2.76

c. Tissue specimens from swine, collected at necropsy, did not contain significant radioactivity, e.g., no specimen registered more than 10 counts per minute (CPM) above background. See Appendix D for individual tissue data in swine.

d. Urinary excretion of ^{14}C in rabbits dermally exposed to radio labeled AI3-37220 appears in Table 3. Significant dermal absorption occurred within the first day of exposure, accounting for over 80 percent of that absorbed through 7 days. Washing the skin site after the first 24 hours recovered only one percent of the applied dose but still reduced the total absorption. When the substance was left on the skin for 7 days, an additional 7 percent was absorbed through the week. Appendices E and F present the individual animal excretion data for the 7-day and 24-hour groups, respectively.

e. A summary of recovered ^{14}C from all sources in rabbits following dermal application of the test substance appears in Table 4. The majority of the unabsorbed radiocarbon was reclaimed from the nonocclusive patch covering the application site at 24 hours. Total

TABLE 3. MEAN (n=6) DAILY URINARY EXCRETION OF ^{14}C FOLLOWING A SINGLE TOPICAL APPLICATION OF ^{14}C-LABELED AI3-37220 TO RABBITS

Exposure Duration	Dose (mg)	Day 1	2	3	4	5	6	7	Total
		Percent of Applied Dose (±SD)							
24 hrs	50	57.6 ±14.2	10.6 ±9.6	0.9 ±0.6	0.2 ±0.1	0.1 ±0.0	0.2 ± 0.2	0.1 ±0.1	69.7 ±15.8
7 days	50	68.0 ±9.9	4.6 ±2.9	2.6 ±2.8	0.5 ±0.4	0.3 ±0.1	0.2 ±0.2	0.2 ±0.1	76.4 ±7.3

TABLE 4. MEAN (n=6) TOTAL RECOVERY OF ^{14}C IN RABBITS THROUGH 7 DAYS FOLLOWING A SINGLE TOPICAL APPLICATION OF ^{14}C-LABELED AI3-37220

Exposure Duration	Dose (mg)	Urine	24 hr Wash	24 hr Patch	7 da Patch	7 da Skin Appl Site	Total
		Percent of Applied Dose (±SD)					
24 hours	50	69.7 ±15.8	1.0 ±0.4	16.3 ±1.3	0.4 ±0.2	0.1 ±0.1	87.6 ±16.1
7 days	50	76.4 ±7.3	---- ----	16.8 ±6.0	0.9 ±0.3	0.2 ±0.1	94.4 ±4.9

accountability was 94 percent in rabbits exposed for the entire 7 day test, and 88 percent in animals which had the material removed after the first day. Appendices G and H provide the individual mass balance data for rabbits exposed for 7 days and 24 hours, respectively.

f. Tissue specimens harvested at necropsy from rabbits did not contain significant radiocarbon. No specimen measured greater than 10 counts per minute (CPM) above background ^{14}C (see Appendix I).

g. The efficiency of application, as measured by delivering a 0.5 mL dose of ^{14}C-labeled AI3-37220 into a flask containing methanol, indicated that swine received 94 percent of the intended dose (n=5). In rabbits, 100 percent of each intended dose were delivered (n=6).

7. DISCUSSION.

a. The domestic pig was used to assess the dermal penetration of AI3-37220 because it reasonably simulates man's absorption kinetics. While no animal model is the perfect human surrogate, the pig, and perhaps the rhesus monkey, are the species of choice for in vivo skin absorption testing (references 3 and 4). Carver and Riviere (reference 5) reviewed the dermatological and physiochemical characteristics of pig skin and found striking similarities to that of man. Several other investigators have reported comparable skin absorption rates and penetration characteristics between the pig and man for a wide range of chemical substances (references 6-10). The rabbit was also used in the present study because it maximizes percutaneous absorption of xenobiotics compared to the other mammalian species, including man (references 3 and 4). Reportedly, 5 to 15 fold increases in absorption are not unusual between man and rabbit. The rabbit does, however, afford a means of evaluating the retention of chemicals within the animal body at levels higher than would be attained in humans. For predictive purposes, human absorption of topical substances is uniformly less than that measured in animals, regardless of species (references 6-10). Accordingly, it provides an additional margin of safety when predicting human risk from dermal exposure based upon animal data.

b. The dose applied to each animal, rabbits or pigs, was based upon an estimate of the application rate recommended for the insect repellent Deet (N,N-diethyl-m-toluamide). This is the standard military skin repellent and contains 33 percent Deet as the active ingredient (AI). The directions call for a total application of about 7.5 mL. This equals 2400 mg of the AI. The usual sites of application are the head, neck, arms and hands which together account for about 0.506 m^2 of skin surface (reference 11). Accordingly, 2400 mg $\div$ 0.506 m^2 = 0.5 mg/cm^2; the dose rate used in the present study in both swine and rabbits.

c. It appears from the rabbit data that there is not a great difference in absorption as a consequence of leaving the substance on the skin for 7 days or removing it after 24 hours. In fact, washing the skin after 24 hours may enhance penetration during the second day as evidenced in both rabbits and swine. It was reported earlier that washing the skin after topical exposure to the drug hydrocortisone, the insecticide malathion, or PCBs increased overall absorption by as much as 100 percent (reference 12). It is possible that the unabsorbed fraction is redistributed on the skin surface by the act of washing, or that the abrasive effects of the process disrupt the epidermal barrier.

d. Based upon the observed data in the present study, and the known comparative absorption rates between man and swine, it is predicted that percutaneous penetration of applied AI3-37220 in humans would be less than 8 percent of the applied dose. The substance, once absorbed, would be rapidly eliminated by urinary excretion, generally within 24 hours. It is unlikely that elimination via feces or by the respiratory route would be significant since nearly all (98 percent) of the measured radioactivity was accounted for in the swine studies. Neither was any notable radiocarbon detected in any separate organ or tissue system of rabbits or swine. Evaporation of AI3-37220 from the skin surface may reach 20 percent of the applied dose within 24 hours, based upon measurements of radiocarbon recovered in the nonocclusive patches of rabbits and swine.

8. CONCLUSIONS. The candidate insect repellent AI3-37220 is minimally absorbed in swine following topical application at a rate of 0.5 mg/cm^2. In rabbits, marked absorption occurs through 1 week. Urinary excretion is the primary elimination pathway of absorbed chemical. Metabolic elimination of AI3-37220 (or its metabolites) is rapid, occurring within 24 hours of absorption. No potential for bioaccumulation has been demonstrated in animals following exposure at 0.5 mg/cm^2. Within the intended use of AI3-37220 as a topical insect repellent, skin absorption in humans would be expected to be less than 8 percent of the applied dose.

Evaporation of AI3-37220 from the skin surface is likely to exceed 20 percent of the applied dose within the first day of exposure.

HUBERT L. SNODGRASS
Biologist
Master Consultant
Toxicity Evaluation Program

JOHN T. HOUPT
Biologist
Toxicity Evaluation Program

PATRICIA A. BEALL
Biological Scientific Laboratory
Technologist
Toxicity Evaluation Program

APPROVED:

14 Nov 96
LEROY W. METKER
Program Manager
Toxicity Evaluation

APPENDIX A

REFERENCES

1. Report. Phase 1, Short Term Toxicity, Toxicological Study No. 75-51-YA80-94, Assessment of the Relative Toxicity of Candidate Insect Repellent AI3-37220, 1-(3-Cyclohexen-1-ylcarbonyl)-2-Methylpiperidine, December 1993. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland.

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APPENDIX B

URINARY EXCRETION OF ^{14}C IN SWINE AFTER A SINGLE DERMAL DOSE OF ^{14}C-AI3-37220

TEST LENGTH -	7 Days	RADIOCARBON APPLIED - uCi	5
VEHICLE -	Acetone	AI3-37220 APPLIED - mg	50
DOSE CORRECTION -	0.938	mg/uCi -	10
BACKGROUND -	22	VOLUME CORRECTION	5
LLD - lower limit detectability	5	cpm/uCi -	2220000

ANIMAL NO.		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	TOTAL
1	URINE VOL-ML	512	880	495	505	550	665	675	
	DPM	211	104	44	31	<LLD	<LLD	<LLD	
	DPM/ML	1,007	437	117	48	0	0	0	
	TOTAL uCi	0.23	0.17	0.03	0.01	0.00	0.00	0.00	
	TOTAL mg	2.32	1.73	0.26	0.11	0.00	0.00	0.00	
	% RECOVERY	4.65	3.47	0.52	0.22	0.00	0.00	0.00	8.85
2	URINE VOL-ML	130	605	490	855	1,075	750	1,160	
	DPM	560	138	39	<LLD	<LLD	<LLD	<LLD	
	DPM/ML	2,868	618	91	0	0	0	0	
	TOTAL uCi	0.17	0.17	0.02	0.00	0.00	0.00	0.00	
	TOTAL mg	1.68	1.69	0.20	0.00	0.00	0.00	0.00	
	% RECOVERY	3.36	3.37	0.40	0.00	0.00	0.00	0.00	7.13
3	URINE VOL-ML	560	950	220	420	465	695	410	
	DPM	166	85	83	36	58	57	<LLD	
	DPM/ML	768	336	325	75	192	187	0	
	TOTAL uCi	0.19	0.14	0.03	0.01	0.04	0.06	0.00	
	TOTAL mg	1.94	1.44	0.32	0.14	0.40	0.58	0.00	
	% RECOVERY	3.87	2.87	0.64	0.28	0.80	1.17	0.00	9.65
4	URINE VOL-ML	585	755	640	440	565	765	515	
	DPM	171	95	39	34	30	28	<LLD	
	DPM/ML	794	389	91	64	43	32	0	
	TOTAL uCi	0.21	0.13	0.03	0.01	0.01	0.01	0.00	
	TOTAL mg	2.09	1.32	0.26	0.13	0.11	0.11	0.00	
	% RECOVERY	4.19	2.65	0.52	0.25	0.22	0.22	0.00	8.05
5	URINE VOL-ML	590	830	520	600	535	555	630	
	DPM	138	97	50	31	<LLD	<LLD	<LLD	
	DPM/ML	618	400	149	48	0	0	0	
	TOTAL uCi	0.16	0.15	0.03	0.01	0.00	0.00	0.00	
	TOTAL mg	1.64	1.49	0.35	0.13	0.00	0.00	0.00	
	% RECOVERY	3.29	2.99	0.70	0.26	0.00	0.00	0.00	7.23
6	URINE VOL-ML	368	440	370	340	445	625	595	
	DPM	353	166	43	28	<LLD	<LLD	<LLD	
	DPM/ML	1,764	768	112	32	0	0	0	
	TOTAL uCi	0.29	0.15	0.02	0.00	0.00	0.00	0.00	
	TOTAL mg	2.92	1.52	0.19	0.05	0.00	0.00	0.00	
	% RECOVERY	5.85	3.04	0.37	0.10	0.00	0.00	0.00	9.36
MEAN % RECOVERY		4.20	3.06	0.53	0.19	0.17	0.23	0.00	8.38
STANDARD DEVIATION		0.87	0.28	0.12	0.10	0.29	0.43	0.00	0.98

APPENDIX C

RECOVERY OF 14C IN SWINE FOLLOWING A SINGLE TOPICAL APPLICATION OF ^{14}C-LABELED AI3-37220

TEST LENGTH -	7 DAYS	EFFICIENCY -	0.938
VEHICLE -	ACETONE	BACKGROUND -	22.00
RADIOCARBON APPLIE	5.00	LLD -	5.00
AI3-37220 APPLIED - mg	50.00	cpm/uCi -	2220000.00
mg/uCi -	10.00		

ANIMAL NO.		URINE	24 HR WASH	24 HR BINDING	TERM BINDING	TERM SKN APP SITE	TOTAL % RECOVERY
1	DPM/ML		27179	3030	456	715	
	VOL (ML)		225	900	900	300	
	TOTAL uCi		2.75	1.23	0.18	0.10	
	TOTAL mg		27.55	12.28	1.85	0.97	
	% OF APPL	8.85	55.09	24.57	3.70	1.93	94.14
2	DPM/ML		39635	2165	516	951	
	VOL (ML)		200	900	900	300	
	TOTAL uCi		3.57	0.88	0.21	0.13	
	TOTAL mg		35.71	8.78	2.09	1.29	
	% OF APPL	7.13	71.41	17.55	4.18	2.57	102.85
3	DPM/ML		35848	1954	608	1055	
	VOL (ML)		200	900	900	300	
	TOTAL uCi		3.23	0.79	0.25	0.14	
	TOTAL mg		32.30	7.92	2.46	1.43	
	% OF APPL	9.65	64.59	15.84	4.93	2.85	97.87
4	DPM/ML		26946	1408	492	995	
	VOL (ML)		300	900	900	300	
	TOTAL uCi		3.64	0.57	0.20	0.13	
	TOTAL mg		36.41	5.71	1.99	1.34	
	% OF APPL	8.05	72.83	11.42	3.99	2.69	98.97
5	DPM/ML		25271	4519	538	1268	
	VOL (ML)		200	900	900	300	
	TOTAL uCi		2.28	1.83	0.22	0.17	
	TOTAL mg		22.77	18.32	2.18	1.71	
	% OF APPL	7.23	45.53	36.64	4.36	3.43	97.19
6	DPM/ML		34889	1403	242	786	
	VOL (ML)		225	900	900	300	
	TOTAL uCi		3.54	0.57	0.10	0.11	
	TOTAL mg		35.36	5.69	0.98	1.06	
	% OF APPL	9.36	70.72	11.38	1.96	2.12	95.54
MEAN % RECOVERY		8.38	63.36	19.57	3.85	2.60	97.76
STANDARD DEVIATIO		0.98	9.97	8.83	0.93	0.49	2.76

APPENDIX D

^{14}C REMAINING IN TISSUES 7 DAYS AFTER DERMAL APPLICATION OF ^{14}C-LABELED AI3-37220 IN SWINE

Animals 1-3	Animals 4-6
% Counting Efficiency - 87.24	% Counting Efficiency - 73.70
% Counting Recovery - 99.10	% Chemical Recovery - 100.00
Background - 30.25	Background - 29.06
Avg Sample Weight - 0.5g	Avg Sample Weight - 0.5g
LLD - avg 15 dpm/g	LLD - avg 18 dpm/g

	Animal Number							
Specimen	1	2	3	4	5	6	MEAN	S.D.
	Activity (dpm/g)							
Bone	1	10	4	7	29	1	8.7	9.6
Bone Marrow	7	5	5	11	6	4	6.3	2.3
Brain	0	0	3	9	4	3	3.2	3
Fat	14	0	8	25	1	16	10.7	8.7
Heart	1	0	0	2	3	13	3.2	4.5
Kidney	0	0	0	8	16	0	4	6.1
Liver	1	0	4	4	8	5	4	2.2
Lungs	6	2	0	4	7	5	3.7	2.7
Muscle	0	0	3	7	0	3	2.2	2.5
Spleen	0	0	2	1	3	5	1.8	1.8
Adrenal Glands	0	0	5	7	6	2	3.3	2.8
Thyroid Glands	0	5	3	11	6	9	5.7	3.6
Urinary Bladder	0	2	0	0	0	4	1	1.5
Skin - Normal	1	1	1	17	9	5	5.7	5.8
Whole Blood	0	0	0	0	0	0	0	0

APPENDIX E

URINARY EXCRETION OF ^{14}C IN RABBITS AFTER A SINGLE DERMAL DOSE OF ^{14}C-AI3-37220 (7-DAY EXPOSURE)

TEST LENGTH -	7 Days	RADIOCARBON APPLIED - uCi	5
VEHICLE -	Acetone	AI3-37220 APPLIED - mg	50
EFFICIENCY -	1.000	mg/uCi -	10
BACKGROUND -	27	VOLUME CORRECTION	5
LLD - lower limit detectability	5	cpm/uCi -	2220000

ANIMAL NO.		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	TOTAL
462	URINE VOL-ML	246	77	258	398	64	144	250	
	DPM	4885	1961	562	101	222	120	63	
	DPM/ML	24290	9670	2675	370	975	465	180	
	TOTAL uCi	2.69	0.34	0.31	0.07	0.03	0.03	0.02	
	TOTAL mg	26.92	3.35	3.11	0.66	0.28	0.30	0.20	
	% RECOVERY	53.83	6.71	6.22	1.33	0.56	0.60	0.41	69.65
504	URINE VOL-ML	138	148	136	63	106	90	77	
	DPM	12685	669	104	90	67	47	54	
	DPM/ML	63290	3210	385	315	200	100	135	
	TOTAL uCi	3.93	0.21	0.02	0.01	0.01	0.00	0.00	
	TOTAL mg	39.34	2.14	0.24	0.09	0.10	0.04	0.05	
	% RECOVERY	78.68	4.28	0.47	0.18	0.19	0.08	0.09	83.98
464	URINE VOL-ML	130	5	176	142	166	228	101	
	DPM	12810	1130	884	70	61	50	43	
	DPM/ML	63915	5515	4285	215	170	115	80	
	TOTAL uCi	3.74	0.01	0.34	0.01	0.01	0.01	0.00	
	TOTAL mg	37.43	0.12	3.40	0.14	0.13	0.12	0.04	
	% RECOVERY	74.86	0.25	6.79	0.28	0.25	0.24	0.07	82.74
465	URINE VOL-ML	202	165	182	119	88	136	88	
	DPM	7345	1226	179	113	67	48	48	
	DPM/ML	36590	5995	760	430	200	105	105	
	TOTAL uCi	3.33	0.45	0.06	0.02	0.01	0.01	0.00	
	TOTAL mg	33.29	4.46	0.62	0.23	0.08	0.06	0.04	
	% RECOVERY	66.59	8.91	1.25	0.46	0.16	0.13	0.08	77.58
466	URINE VOL-ML	80	54	88	35	80	124	172	
	DPM	21514	905	158	137	95	51	43	
	DPM/ML	107435	4390	655	550	340	120	80	
	TOTAL uCi	3.87	0.11	0.03	0.01	0.01	0.01	0.01	
	TOTAL mg	38.72	1.07	0.26	0.09	0.12	0.07	0.06	
	% RECOVERY	77.43	2.14	0.52	0.17	0.25	0.13	0.12	80.76
467	URINE VOL-ML	112	131	122	165	140	220	182	
	DPM	11194	956	141	125	69	52	40	
	DPM/ML	55835	4645	570	490	210	125	65	
	TOTAL uCi	2.82	0.27	0.03	0.04	0.01	0.01	0.01	
	TOTAL mg	28.17	2.74	0.31	0.36	0.13	0.12	0.05	
	% RECOVERY	56.34	5.48	0.63	0.73	0.26	0.25	0.11	63.79
MEAN % RECOVERY		67.95	4.63	2.65	0.52	0.28	0.24	0.15	76.42
STANDARD DEVIATION		9.91	2.86	2.75	0.41	0.13	0.17	0.12	7.33

APPENDIX F

URINARY EXCRETION OF ^{14}C IN RABBITS AFTER A SINGLE DERMAL DOSE OF ^{14}C-AI3-37220*
(24-HOUR EXPOSURE)

TEST LENGTH -	7 Days	RADIOCARBON APPLIED - uCi	5
VEHICLE -	Acetone	AI3-37220 APPLIED - mg	50
EFFICIENCY -	1.000	mg/uCi -	10
BACKGROUND -	27	VOLUME CORRECTION	5
LLD - lower limit detectability	5	cpm/uCi -	2220000

ANIMAL NO.		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	TOTAL
468	URINE VOL-ML	65	80	72	64	70	65	144	
	DPM	25204	872	161	99	70	70	42	
	DPM/ML	125885	4225	670	360	215	215	75	
	TOTAL uCi	3.69	0.15	0.02	0.01	0.01	0.01	0.00	
	TOTAL mg	36.86	1.52	0.22	0.10	0.07	0.06	0.05	
	% RECOVERY	73.72	3.05	0.43	0.21	0.14	0.13	0.10	77.76
469	URINE VOL-ML	154	120	142	81	156	138	90	
	DPM	8972	3274	336	107	50	60	43	
	DPM/ML	44725	16235	1545	400	115	165	80	
	TOTAL uCi	3.10	0.88	0.10	0.01	0.01	0.01	0.00	
	TOTAL mg	31.03	8.78	0.99	0.15	0.08	0.10	0.03	
	% RECOVERY	62.05	17.55	1.98	0.29	0.16	0.21	0.06	82.30
470	URINE VOL-ML	115	161	89	80	70	228		
	DPM	9832	4027	304	108	66	72		
	DPM/ML	49025	20000	1385	405	195	225		
	TOTAL uCi	2.54	1.45	0.06	0.01	0.01	0.02		
	TOTAL mg	25.40	14.50	0.56	0.15	0.06	0.23		
	% RECOVERY	50.79	29.01	1.11	0.29	0.12	0.46		81.79
471	URINE VOL-ML	144	178	198	211	232	248	136	
	DPM	11581	471	92	51	40	33	31	
	DPM/ML	57770	2220	325	120	65	30	20	
	TOTAL uCi	3.75	0.18	0.03	0.01	0.01	0.00	0.00	
	TOTAL mg	37.47	1.78	0.29	0.11	0.07	0.03	0.01	
	% RECOVERY	74.94	3.56	0.58	0.23	0.14	0.07	0.02	79.54
472	URINE VOL-ML	36	100	74	66	67	12	124	
	DPM	30378	1541	120	68	57	31	43	
	DPM/ML	151755	7570	465	205	150	20	80	
	TOTAL uCi	2.46	0.34	0.02	0.01	0.00	0.00	0.00	
	TOTAL mg	24.61	3.41	0.16	0.06	0.05	0.00	0.04	
	% RECOVERY	49.22	6.82	0.31	0.12	0.09	0.00	0.09	56.65
473	URINE VOL-ML	73	71	138	94	67	90	220	
	DPM	10599	1231	149	88	57	67	61	
	DPM/ML	52860	6020	610	305	150	200	170	
	TOTAL uCi	1.74	0.19	0.04	0.01	0.00	0.01	0.02	
	TOTAL mg	17.38	1.93	0.38	0.13	0.05	0.08	0.17	
	% RECOVERY	34.76	3.85	0.76	0.26	0.09	0.16	0.34	40.22
MEAN % RECOVERY		57.58	10.64	0.86	0.23	0.12	0.17	0.10	69.71
STANDARD DEVIATION		14.25	9.61	0.56	0.06	0.03	0.15	0.11	15.84

* GROUP A - Substance removed after 24 hours.

APPENDIX G

RECOVERY OF ^{14}C IN RABBITS AFTER A SINGLE TOPICAL APPLICATION OF ^{14}C-LABELED AI3-37220 (7-DAY EXPOSURE)

TEST LENGTH -	7 DAYS	EFFICIENCY -	1.00
VEHICLE -	ACETONE	BACKGROUND -	27.00
RADIOCARBON APPLIE	5.00	VOL CORRECTION-	2.00
AI3-37220 APPLIED - mg	50.00	LLD -	5.00
mg/uCi -	10.00	cpm/uCi -	2220000.00

ANIMAL NO.		URINE	24 HR WASH	24 HR BINDING	TERM BINDING	TERM SKN APP SITE	TOTAL % RECOVERY
462	DPM			5286	167	201	
	DPM/ML			10518	280	348	
	VOL (ML)			300	300	100	
	TOTAL uCi			1.42	0.04	0.02	
	TOTAL mg			14.21	0.38	0.16	
	% OF APPL	69.65	N/P	28.43	0.76	0.31	99.15
504	DPM			2164	165	179	
	DPM/ML			4274	276	304	
	VOL (ML)			300	300	100	
	TOTAL uCi			0.58	0.04	0.01	
	TOTAL mg			5.78	0.37	0.14	
	% OF APPL	83.98	N/P	11.55	0.75	0.27	96.55
464	DPM			2309	215	83	
	DPM/ML			4564	376	112	
	VOL (ML)			300	300	100	
	TOTAL uCi			0.62	0.05	0.01	
	TOTAL mg			6.17	0.51	0.05	
	% OF APPL	82.74	N/P	12.34	1.02	0.10	96.19
465	DPM			3333	280	133	
	DPM/ML			6612	506	212	
	VOL (ML)			300	300	100	
	TOTAL uCi			0.89	0.07	0.01	
	TOTAL mg			8.94	0.68	0.10	
	% OF APPL	77.58	N/P	17.87	1.37	0.19	97.01
466	DPM			2200	109	109	
	DPM/ML			4346	164	164	
	VOL (ML)			300	300	100	
	TOTAL uCi			0.59	0.02	0.01	
	TOTAL mg			5.87	0.22	0.07	
	% OF APPL	80.76	N/P	11.75	0.44	0.15	93.10
467	DPM			3546	237	123	
	DPM/ML			7038	420	192	
	VOL (ML)			300	300	100	
	TOTAL uCi			0.95	0.06	0.01	
	TOTAL mg			9.51	0.57	0.09	
	% OF APPL	63.79	N/P	19.02	1.14	0.17	84.12
MEAN % RECOVERY		76.42		16.83	0.91	0.20	94.35
STANDARD DEVIATION		7.33		5.98	0.30	0.07	4.91

N/P - Not performed

APPENDIX H

RECOVERY OF ^{14}C IN RABBITS AFTER A SINGLE TOPICAL APPLICATION OF ^{14}C-LABELED AI3-37220 (24-HR EXPOSURE)

TEST LENGTH -	7 DAYS	EFFICIENCY -	1.00
VEHICLE -	ACETONE	BACKGROUND dpm -	27.00
14C APPLIED - uCi	5.00	VOL CORRECTION-	2.00
AI3-37220 APPLIED - mg	50.00	LLD - lower limit detectability	5.00
mg/uCi -	10.00	cpm/uCi -	2220000.00

ANIMAL NO.		URINE	24 HR WASH	24 HR BINDING	TERM BINDING	TERM SKN APP SITE	TOTAL RECOVERY
468	DPM		156	3278	118	57	
	DPM/ML		258	6502	182	60	
	VOL (ML)		100	300	300	100	
	TOTAL uCi		0.01	0.88	0.02	0.00	
	TOTAL mg		0.12	8.79	0.25	0.03	
	% OF APPL	77.76	0.23	17.57	0.49	0.05	96.11
469	DPM		456	2939	146	151	
	DPM/ML		858	5824	238	248	
	VOL (ML)		100	300	300	100	
	TOTAL uCi		0.04	0.79	0.03	0.01	
	TOTAL mg		0.39	7.87	0.32	0.11	
	% OF APPL	82.30	0.77	15.74	0.64	0.22	99.68
470	DPM		788	3443	95	119	
	DPM/ML		1522	6832	136	184	
	VOL (ML)		100	300	300	100	
	TOTAL uCi		0.07	0.92	0.02	0.01	
	TOTAL mg		0.69	9.23	0.18	0.08	
	% OF APPL	81.79	1.37	18.46	0.37	0.17	102.16
471	DPM		741	2758	42	116	
	DPM/ML		1428	5462	30	178	
	VOL (ML)		100	300	300	100	
	TOTAL uCi		0.06	0.74	0.00	0.01	
	TOTAL mg		0.64	7.38	0.04	0.08	
	% OF APPL	79.54	1.29	14.76	0.08	0.16	95.83
472	DPM		562	2818	98	92	
	DPM/ML		1070	5582	142	130	
	VOL (ML)		100	300	300	100	
	TOTAL uCi		0.05	0.75	0.02	0.01	
	TOTAL mg		0.48	7.54	0.19	0.06	
	% OF APPL	56.65	0.96	15.09	0.38	0.12	73.20
473	DPM		672	3054	109	104	
	DPM/ML		1290	6054	164	154	
	VOL (ML)		100	300	300	100	
	TOTAL uCi		0.06	0.82	0.02	0.01	
	TOTAL mg		0.58	8.18	0.22	0.07	
	% OF APPL	40.22	1.16	16.36	0.44	0.14	58.33
MEAN% RECOVERY		69.71	0.96	16.33	0.40	0.14	87.55
STND DEVIATION		15.84	0.38	1.32	0.17	0.05	16.14

APPENDIX I

^{14}C REMAINING IN TISSUES 7 DAYS AFTER DERMAL APPLICATION OF ^{14}C-LABELED AI3-37220 IN RABBITS

Animals 462, 464, 465, 468
% Counting Efficiency - 69.28
% Chemical Recovery - 100.00
Background - 25.72
Average Sample Wt. - 0.5g
LLD (lower limit detectability) - avg 22 dpm/g

Animals 469, 470
% Counting Efficiency - 69.42
% Chemical Recovery - 100.00
Background - 29.96
Average Sample Wt. - 0.5g
LLD - avg 24 dpm/g

Specimen	Animal Number 462*	464*	465*	468	469	470	MEAN	S.D.
	Activity (dpm/g)							
Bone	0	1	8	4	2	0	2.5	2.8
Bone Marrow	20	20	10	7	0	0	9.5	8.2
Brain	10	2	7	2	0	0	3.5	3.7
Fat	30	30	0	8	0	30	16.3	13.9
Heart	1	4	0	10	1	5	3.5	3.4
Kidney	7	2	9	8	20	9	9.2	5.4
Liver	10	10	9	8	6	10	8.8	1.5
Lungs	20	10	7	30	0	10	12.8	9.7
Muscle	6	4	1	7	0	0	3.0	2.8
Spleen	0	20	0	5	0	0	4.2	7.3
Adrenal Glands	30	5	0	0	0	10	7.5	10.7
Thyroid Glands	5	0	10	4	20	10	8.2	6.3
Urinary Bladder	3	4	0	4	7	0	3.0	2.4
Skin - Normal	8	8	0	10	1	4	5.2	3.8
Testes	0	4	4	2	0	1	1.8	1.7

* Test substance remaining on the back for 7 days; removed after 24 hours in remaining rabbits.